



REMARKS

In response to the Notification, attached is a Sequence Listing. Also transmitted herewith is a copy of the Sequence Listing in computer readable form. As required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d) Applicants' Attorney hereby states that the content of the Sequence Listing in paper form and on the computer readable form of the Sequence Listing are the same, and the submission includes no new matter.

A Declaration and Power of Attorney, signed by the inventor will follow in due course.

In the event that there are any fee deficiencies or additional fees are payable, please charge the same or credit any overpayment to our Deposit Account (Account No. 04-0213).

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service, with sufficient postage, as First Class Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on August 24, 2001.

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

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Genetics	
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
PNA	peptide nucleic acid (Synthetic DNA or RNA in which the sugar-phosphate moiety is replaced by an amino acid. If the sugar-phosphate moiety is replaced by the -NH-(CH ₂) ₂ -N(COCH ₂ -base)-CH ₂ CO- moiety, PNA will hybridize with DNA.)
A	adenine
G	guanine
C	cytosine
T	thymine
base	A, G, T, or C
bp	base pair
nucleic acid	At least two covalently joined nucleotides or at least two covalently joined pyrimidine (e.g. cytosine, thymine, or uracil) or purine bases (e.g. adenine or guanine). The term nucleic acid refers to any backbone of the covalently joined pyrimidine or purine bases, such as e.g. to the sugar-phosphate backbone of DNA, cDNA, or RNA, to a peptide backbone of PNA, or to analogous structures (e.g. a phosphoramidate, thiophosphate, or dithiophosphate backbone). The essential feature of a nucleic acid according to the present invention is that it can sequence-specifically bind naturally occurring cDNA or RNA.
nucleic acid oligomer	Nucleic acid of base length that is not further specified (e.g. nucleic acid octamer: a nucleic acid having any backbone in which 8 pyrimidine or purin bases are covalently bound to one another).
oligomer	Equivalent to nucleic acid oligomer.
oligonucleotide	Equivalent to oligomer or nucleic acid oligomer, thus e.g. a DNA, PNA, or RNA fragment of base length that is not further specified.
oligo	Abbreviation for oligonucleotide.
dATP	Deoxyribonucleoside triphosphate of A (DNA moiety with the A base and two further phosphates to build a longer DNA fragment or oligonucleotide).

dGTP	Deoxyribonucleoside triphosphate of G (DNA moiety with the G base and two further phosphates to build a longer DNA fragment or oligonucleotide).
dCTP	Deoxyribonucleoside triphosphate of C (DNA moiety with the C base and two further phosphates to build a longer DNA fragment or oligonucleotide).
dTTP	Deoxyribonucleoside triphosphate of T (DNA moiety with the T base and two further phosphates to build a longer DNA fragment or oligonucleotide).
primer	Initial complementary fragment of an oligonucleotide, with the base length of the primer being only approx. 4-8 bases. Serves as the starting point for enzymatic replication of an oligonucleotide.
mismatch	To form the Watson Crick double-stranded oligonucleotide structure, the two single strands hybridize in such a way that the A (or C) base of one strand forms hydrogen bonds with the T (or G) base of the other strand (in RNA, T is replaced by uracil). Any other base pairing does not form hydrogen bonds, distorts the structure, and is referred to as a "mismatch."
ds	double strand
ss	single strand
<i>Chemical Substances/Groups</i>	
R	A substituent or side chain of any organic residue not further specified.
redox	redox-active substance
alkyl	The term "alkyl" refers to a saturated hydrocarbon radical that is straight-chained or branched (e.g. ethyl, isopropyl, or 2,5-dimethylhexyl, etc.). When "alkyl" is used to indicate a linker or spacer, the term refers to a group having two available valences for covalent linkage (e.g. $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, or $-\text{CH}_2\text{C}(\text{CH}_3)_2\text{CH}_2\text{CH}_2\text{C}(\text{CH}_3)_2\text{CH}_2-$, etc.). Alkyl groups preferred as substituents or side chains R are those of chain length 1-30 (longest continuous chain of atoms bound to one another). Alkyl groups preferred as linkers or spacers are those of chain length 1-20, especially of chain length of 1-14, the chain length representing

	the shortest continuous link between linker or spacer-joined structures.
alkenyl	Alkyl groups in which one or more of the C-C single bonds are replaced by C=C double bonds.
alkinyl	Alkyl or alkenyl groups in which one or more of the C-C single or C=C double bonds are replaced by C≡C triple bonds.
heteroalkyl	Alkyl groups in which one or more of the C-H bonds or C-C single bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkenyl	Alkenyl groups in which one or more C-H bonds, C-C single, or C=C double bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkinyl	Alkinyl groups in which one or more of the C-H bonds, C-C single, C=C double, or C≡C triple bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
linker	A molecular link between two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule. Linkers can usually be purchased in the form of alkyl, alkenyl, alkinyl, heteroalkyl, heteroalkenyl, or heteroalkinyl chains, the chain being derivatized in two places with (identical or different) reactive groups. These groups form a covalent chemical bond in simple/known chemical reactions with the appropriate reaction partner. The reactive groups may also be photoactivatable, i.e. the reactive groups are activated only by light of a specific or random wavelength. Preferred linkers are those of chain length of 1-20, especially of chain length of 1-14, the chain length representing here the shortest continuous link between the structures to be joined, thus between the two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule.
spacer	A linker that is covalently attached via the reactive groups to one or both of the structures to be joined (see linker). Preferred spacers are those of chain length 1-20, especially of chain length 1-14, the chain length representing the shortest continuous link between the structures to be joined.

(n x HS-spacer)-oligo	A nucleic acid oligomer to which n thiol functions are each attached via a spacer, where each spacer may have a different chain length (shortest continuous link between the thiol function and the nucleic acid oligomer), especially any chain length between 1 and 14 each. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been fixed thereto by means of modification, and "n" is any integer, especially a number between 1 and 20.
(n x R-S-S-spacer)-oligo	A nucleic acid oligomer to which n disulfide functions are each attached via a spacer, and any residue R saturates the disulfide function. Each spacer for attaching the disulfide function to the nucleic acid oligomer may have a different chain length (shortest continuous link between the disulfide function and the nucleic acid oligomer), especially any chain length between 1 and 14 each. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been fixed thereto by means of modification. The placeholder "n" is any integer, especially a number between 1 and 20.
oligo-spacer-S-S-spacer-oligo	Two identical or different nucleic acid oligomers that are joined to each other via a disulfide bridge, the disulfide bridge being attached to the nucleic acid oligomers via any two spacers and the two spacers potentially having differing chain lengths (shortest continuous link between the disulfide bridge and the respective nucleic acid oligomer), especially any chain length between 1 and 14 each, and these spacers, in turn, potentially being bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been fixed thereto by means of modification.
PQQ	pyrroloquinoline quinone; corresponds to 4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-f]-quinoline-2,7,9-tricarboxylic acid)
TEATFB	tetraethylammonium-tetrafluoroborate
sulfo-NHS	N-hydroxysulfosuccinimide
EDC	(3-dimethylaminopropyl)-carbodiimide

HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
Tris	trishydroxymethylamino methane
EDTA	ethylenediamine tetraacetate (sodium salt)
cystamine	(H ₂ N-CH ₂ -CH ₂ -S-) ₂
Modified Surfaces/Electrodes	
mica	Muskovite platelets, a support for the application of thin layers.
<i>Au-S-ss-oligo-PQQ</i>	Gold film on mica having a covalently applied monolayer of derivatized 12-bp single-strand oligonucleotide (sequence: TAGTCGGAAGCA) SEQ ID NO: 1 . Here, the terminal phosphate group of the oligonucleotide at the 3' end is esterified with (HO-(CH ₂) ₂ -S) ₂ to P-O-(CH ₂) ₂ -S-S-(CH ₂) ₂ -OH, homolytically cleaving the S-S bond and producing one Au-S-R bond each. The terminal thymine base at the 5' end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH ₂ -CH ₂ -NH ₂ and the residue, in turn, is joined via its free amino group with a carboxylic-acid group of the PQQ by means of amidation.
<i>Au-S-ds-oligo-PQQ</i>	Au-S-ss-oligo-PQQ that is hybridized with the oligonucleotide complementary to the ss-oligo (sequence: TAGTCGGAAGCA SEQ ID NO: 1).
Electrochemistry	
<i>E</i>	The electrode potential on the working electrode.
<i>E</i> ₀	Half-wave potential, the potential in the middle between the current maximums for oxidation and reduction of cyclic voltammetrically reversible electrooxidation or reduction.
<i>i</i>	current density (current per cm ² of electrode surface)
cyclic voltammetry	Recording a current-voltage curve. The potential of a stationary working electrode is changed linearly as a function of time, starting at a potential at which no electrooxidation or reduction occurs, up to a potential at which a species that is solute or adsorbed on the electrode is oxidized or reduced (i.e. current flows). After running through the oxidation or reduction operation, which produces in the current-voltage curve an initially increasing current and, after reaching a maximum, a gradually decreasing current, the direction of the potential feed is reversed. The behavior of the products of

	electrooxidation or electroreduction is then recorded in reverse run.
amperometry	Recording a current-time curve. Here, the potential of a stationary working electrode is set, e.g. by means of a potential jump, to a potential at which the electrooxidation or reduction of a solute or adsorbed species occurs, and the flowing current is recorded as a function of time.

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Fig. 1	Shows a schematic illustration of the Sanger method of oligonucleotide sequencing;
Fig. 2	Shows a schematic illustration of oligonucleotide sequencing by means of hybridization on a chip;
Fig. 3	Shows a schematic illustration of the surface hybrid of the general structure elec-spacer-ss-oligo-spacer-redox with a 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' <u>SEQ ID NO: 1</u> (left) and Au-S-ss-oligo-PQQ in the hybridized state as an embodiment example of an elec-spacer-ss-oligo-spacer-redox; only a portion of the probe oligonucleotide having a hybridized complementary strand is shown (right), the attachment of the oligonucleotide to the surface redox-active substance PQQ occurred via the spacer -CH ₂ -CH=CH-CO-NH-CH ₂ -CH ₂ -NH-;
Fig. 4	Shows a cyclic voltammogram of a test site consisting of Au-S-ss-oligo-PQQ (dotted) compared with an identical test site with completely hybridized target (Au-S-ds-oligo-PQQ, solid line);
Fig. 5	Shows a cyclic voltammogram of a test site with completely hybridized target (Au-S-ds-oligo-PQQ) (solid line) compared with a test site with hybridized target that exhibits 2 base-pair mismatches (Au-S-ds-oligo-PQQ with 2 bp mismatches, broken).

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To prepare the ds oligonucleotide solution, a double-modified 12-bp single-strand oligonucleotide of the sequence 5'-TAGTCGGAAGCA-3' SEQ ID NO: 1 was used, which is esterified with $(\text{HO}-(\text{CH}_2)_2-\text{S})_2$ at the phosphate group of the 3' end to $\text{P-O}-(\text{CH}_2)_2-\text{S-S}-(\text{CH}_2)_2-\text{OH}$. At the 5' end, the terminal base of the oligonucleotide, thymine, is modified at the C-5 carbon with $-\text{CH}=\text{CH}-\text{CO}-\text{NH}-\text{CH}_2-\text{CH}_2-\text{NH}_2$. A 2×10^{-4} molar solution of this oligonucleotide in the hybridization buffer (10 mM Tris, 1 mM EDTA, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) was hybridized with a 2×10^{-4} molar solution of the (unmodified) complementary strand in the hybridization buffer at room temperature for approx. 2 hours (hybridization step). During a reaction time of approx. 12-24 h, the disulfide spacer $\text{P-O}-(\text{CH}_2)_2-\text{S-S}-(\text{CH}_2)_2-\text{OH}$ of the oligonucleotide was homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with the Au atoms of the surface, thus causing to a 1:1 coadsorption of the ds-oligonucleotide and the 2-hydroxy-mercaptoethanol.

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Example 2: Producing the Au-S-ss-oligo-PQQ oligonucleotide electrode. Analogously to the production of the Au-S-ds-oligo-PQQ system, the support surface is derivatized with modified single-strand oligonucleotide, dispensing with only the hybridization of the modified oligonucleotide of the sequence 5'-TAGTCGGAAGCA-3' SEQ ID NO: 1 with its complementary strand and, in the incubation step, using only the double-modified 12-bp single-strand probe oligonucleotide (see Example 1) in the form of a 1×10^{-4} molar solution in water and in the presence of 10^{-2} molar Tris, 10^{-3} molar EDTA and 0.7 molar TEATFB (or 1 molar NaCl) at pH 7.5. The redox step was carried out as indicated in Example 1.

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Example 3: Producing the Au-S-ds-oligo-PQQ oligonucleotide electrode having 2 bp mismatches. The production of a support surface derivatized with modified double-strand oligonucleotide was carried out analogously to the production of the Au-S-ds-oligo-PQQ system, but only in hybridizing the modified oligonucleotide of the sequence 5'-TAGTCGGAAGCA-3' SEQ ID NO: 1 was a complementary strand used (sequence: 5'-ATCAG**A**TTTCGT-3') SEQ ID NO: 2, in which bases no. 6 and 7 (counted from the 5' end), which are actually complementary, were modified from C to **A** or from C to **T** to introduce two base-pair mismatches.